RNF186 is differentially expressed in brain metastatic breast cancer.

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Breast cancer patients diagnosed with metastasis to the brain¹⁻³ are presented limited treatment options^{4,5}. We mined published microarray data^{6,7} to discover genes associated with brain metastasis in human breast cancer, and identified differential expression of RNF186 when comparing brain metastatic tissues to primary tumors of the breast. RNF186 was expressed at significantly lower levels in metastasis to the brain as compared to primary tumors. Molecular functions and down-regulation of RNF186, a ring finger E3 ligase that can modulate endoplasmic reticulum-stress pathway signaling⁸ may be relevant to pathways underlying metastasis to the brain in human breast cancer.

Keywords: breast cancer, RNF186, brain metastasis, central nervous system metastasis, systems biology of breast cancer, targeted therapeutics in breast cancer.

A study of patients treated with treated with trastuzumab, a monoclonal antibody targeting the human epidermal growth factor receptor 2 (HER2), reported a rate of metastasis to the central nervous system of 34%². The severity of disease and limited treatment options demand an enhanced understanding of the fundamental transcriptional nature of metastasis to the brain in patients with breast cancer. We mined published microarray data^{6,7} generated using the primary tumors and brain metastatic tissues of patients with human breast cancer to identify genes whose expression was most quantitatively different in brain metastatic tissues. These analyses revealed significant differential expression of RNF186 in metastases to the brain in patients with human breast cancer.

Methods

We used datasets GSE52604⁶ and GSE43837⁷ and for this differential gene expression analysis of brain metastasis in human breast cancer in conjunction with GEO2R. GSE52604 was generated using Whole Human Genome Microarray 4x44K G4112F technology with $n=10$ normal breast tissues and $n=35$ brain metastases from patients with breast cancer, analysis performed using platform GPL6480. GSE43837 was generated using Affymetrix Human X3P Array technology with $n=19$ primary breast tumors and $n=19$ brain metastases from patients with breast cancer, analysis performed using platform GPL1352. The Benjamini and Hochberg method of p -value adjustment was used for ranking of differential expression but raw p -values were used to assess statistical significance of global differential expression. Log-transformation of data was auto-detected, and the NCBI generated category of platform annotation was used. A statistical test was performed to evaluate whether RNF186 gene expression was significantly between primary breast tumors and brain metastases in humans with breast cancer using a two-tailed, unpaired t-test with Welch's correction. We used PRISM for all statistical analyses of differential gene expression in human breast cancer (Version 8.4.0)(455).

Results

We performed comparative transcriptome analysis of primary and metastatic tumor tissues from patients with breast cancer using published microarray data^{6,7} to describe the transcriptional landscape of brain metastasis in human breast cancer and for the identification of potential therapeutic targets.

RNF186 is differentially expressed in the brain metastases of patients with metastatic breast cancer when compared to tissues of the breast, both benign and transformed.

We identified the gene encoding the ring finger 186, RNF186, as among the genes whose expression was most significantly different in the brain metastatic tissues of patients with breast cancer as compared to normal breast tissues. When sorting each of the genes expressed in brain metastases based on significance of change in expression as compared to benign breast tissue, RNF186 ranked 4 out of 22277 total transcripts (Table 1), equating to 99.98% differential expression. Differential expression of RNF186 in metastases to the brain was statistically significant (Table 1; $p=1.29E-25$).

We mined a second microarray dataset to validate differential expression of RNF186 in brain metastatic breast cancer. We again identified RNF186 as among the genes whose expression was most different in the brain metastatic tissues of patients with breast cancer as compared to primary tumors of the breast. When sorting each of the genes expressed in brain metastases based on significance of change in expression as compared to primary tumors of the breast, in this dataset, RNF186 ranked 6132 out of 61359 total transcripts (Table 2), equating to 90.0% differential expression. Differential expression of RNF186 in metastases to the brain was again statistically significant (Table 2; $p=0.04659941$).

RNF186 is expressed at significantly lower levels in metastases to the brain as compared to primary tumors of the breast.

We obtained exact mRNA expression values for RNF186 from normal breast tissue and from brain metastases of patients with breast cancer to evaluate direction and statistical significance of change in RNF186 mRNA expression in metastasis to the brain in human metastatic breast cancer. RNF186 was expressed at lower levels in metastasis to the brain as compared to normal breast tissue (Figure 1), and this difference was statistically significant (Figure 1; $p < 0.0001$).

Thus, through blinded, systems-level analysis of published microarray datasets^{6,7}, we found that RNF186 was among the genes whose expression was most significantly different in the brain metastases of patients with metastatic breast cancer as compared to primary tumors of the breast, and that RNF186 expression was significantly lower in brain metastatic tissues as compared to normal breast tissues and primary tumors of the breast.

Discussion

We provided evidence here that the ring finger protein 186, RNF186, was among the genes most differentially expressed in metastases to the brain as compared to either normal breast tissue or primary tumors of the breast. RNF186 was expressed at significantly lower levels in brain metastatic tissues as compared to primary tumors of the breast. Molecular functions of RNF186, an E3 ring finger ligase that can exert influence over the endoplasmic reticulum (ER)-stress response pathway through its ability to interact with BNip1, a pro-apoptotic, Bcl-2 interacting protein⁸, may be of relevance to the processes which control metastasis of tumor cells from the breast to the brain.

References

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Rank	ID	p-value	t	B	Gene	Gene name
4	A_23_P126248	1.29E-25	-21.1239046	46.632631	RNF186	ring finger protein 186

Table 1: Differential expression of RNF186 in brain metastatic tissues as compared to normal breast tissues in patients with breast cancer.

Rank of differential expression, probe ID, *p*-value with respect to differential expression, *t*, a moderated *t*-statistic, *B*, the log-odds of differential expression between the two groups compared, fold change (FC) of RNF186 expression between the brain metastases of patients with breast cancer and normal tissues of the breast, gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	Gene	Gene name
6132	g9506662_3p_at	0.04659941	-2.049175	-3.83625	RNF186	ring finger protein 186

Table 2: Differential expression of RNF186 in brain metastatic tissues as compared to primary tumors in patients with breast cancer.

Rank of differential expression, probe ID, *p*-value with respect to differential expression, *t*, a moderated *t*-statistic, *B*, the log-odds of differential expression between the two groups compared, gene and gene name are listed in this chart.

Figure 1: RNF186 is expressed at lower levels in brain metastases as compared to normal tissues of the breast in patients with metastatic breast cancer.

The mRNA expression level of RNF186 in benign breast tissue (left) and in the brain metastases of brain metastatic breast cancer patients (right) is graphically represented here with mean RNF186 levels marked and the result of a statistical test evaluating difference in mRNA expression level between brain metastases and normal tissues of the breast, a *p*-value, listed above.